Evaluation of a mosaic HIV-1 vaccine in a multicentre, randomised, double-blind, placebo-controlled, phase 1/2a clinical trial (APPROACH) and in rhesus monkeys (NHP 13-19)

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See **Comment** page 192

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Summary

Background More than 1·8 million new cases of HIV-1 infection were diagnosed worldwide in 2016. No licensed prophylactic HIV-1 vaccine exists. A major limitation to date has been the lack of direct comparability between clinical trials and preclinical studies. We aimed to evaluate mosaic adenovirus serotype 26 (Ad26)-based HIV-1 vaccine candidates in parallel studies in humans and rhesus monkeys to define the optimal vaccine regimen to advance into clinical efficacy trials.

Methods We conducted a multicentre, randomised, double-blind, placebo-controlled phase 1/2a trial (APPROACH). Participants were recruited from 12 clinics in east Africa, South Africa, Thailand, and the USA. We included healthy, HIV-1-uninfected participants (aged 18–50 years) who were considered at low risk for HIV-1 infection. We randomly assigned participants to one of eight study groups, stratified by region. Participants and investigators were blinded to the treatment allocation throughout the study. We primed participants at weeks 0 and 12 with Ad26.Mos.HIV (5×10^{10} viral particles per 0·5 mL) expressing mosaic HIV-1 envelope (Env)/Gag/Pol antigens and gave boosters at weeks 24 and 48 with Ad26.Mos.HIV or modified vaccinia Ankara (MVA; 10^8 plaque-forming units per 0·5 mL) vectors with or without high-dose (250 μg) or low-dose (50 μg) aluminium adjuvanted clade C Env gp140 protein. Those in the control group received 0·9% saline. All study interventions were administered intramuscularly. Primary endpoints were safety and tolerability of the vaccine regimens and Env-specific binding antibody responses at week 28. Safety and immunogenicity were also assessed at week 52. All participants who received at least one vaccine dose or placebo were included in the safety analysis; immunogenicity was analysed using the per-protocol population. We also did a parallel study in rhesus monkeys (NHP 13-19) to assess the immunogenicity and protective efficacy of these vaccine regimens against a series of six repetitive, heterologous, intrarectal challenges with a rhesus peripheral blood mononuclear cell-derived challenge stock of simian-human immunodeficiency virus (SHIV-SF162P3). The APPROACH trial is registered with ClinicalTrials.gov, number NCT02315703.

Findings Between Feb 24, 2015, and Oct 16, 2015, we randomly assigned 393 participants to receive at least one dose of study vaccine or placebo in the APPROACH trial. All vaccine regimens demonstrated favourable safety and tolerability. The most commonly reported solicited local adverse event was mild-to-moderate pain at the injection site (varying from 69% to 88% between the different active groups *vs* 49% in the placebo group). Five (1%) of 393 participants reported at least one grade 3 adverse event considered related to the vaccines: abdominal pain and diarrhoea (in the same participant), increased aspartate aminotransferase, postural dizziness, back pain, and malaise. The mosaic Ad26/Ad26 plus high-dose gp140 boost vaccine was the most immunogenic in humans; it elicited Env-specific binding antibody responses (100%) and antibody-dependent cellular phagocytosis responses (80%) at week 52, and T-cell responses at week 50 (83%). We also randomly assigned 72 rhesus monkeys to receive one of five different vaccine regimens or placebo in the NHP 13-19 study. Ad26/Ad26 plus gp140 boost induced similar magnitude, durability, and phenotype of immune responses in rhesus monkeys as compared with humans and afforded 67% protection against acquisition of SHIV-SF162P3 infection (two-sided Fisher's exact test p=0·007). Env-specific ELISA and enzyme-linked immunospot assay responses were the principal immune correlates of protection against SHIV challenge in monkeys.

Interpretation The mosaic Ad26/Ad26 plus gp140 HIV-1 vaccine induced comparable and robust immune responses in humans and rhesus monkeys, and it provided significant protection against repetitive heterologous SHIV challenges in rhesus monkeys. This vaccine concept is currently being evaluated in a phase 2b clinical efficacy study in sub-Saharan Africa (NCT03060629).

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Introduction

Despite the success of antiretroviral therapy for both treatment and prevention of HIV-1 infection,[1–4] a safe and effective vaccine will most likely be needed to achieve a practical and durable end to the global HIV-1 pandemic.[5,6] However, the challenges associated with the development of an HIV-1 vaccine are unprecedented. Key scientific hurdles include the extensive genetic diversity of the virus, the rapid establishment of latent viral reservoirs, and the unclear immune correlates of protection.[7,8]

To date, four HIV-1 vaccine concepts have been evaluated for efficacy in humans. Clinical efficacy studies with HIV-1 envelope (Env) gp120 subunit vaccines,[9,10] adenovirus serotype 5 (Ad5) vectors expressing the internal proteins Gag/Pol/Nef,[11,12] and a DNA vaccine prime with an Ad5 vector boost[13] did not prevent acquisition of HIV-1 infection in the populations studied. By contrast, a canarypox ALVAC vector prime with an Env gp120 boost provided 31% vaccine efficacy in a study in Thailand,[14] and a clade C version of this vaccine is currently being evaluated in South Africa (NCT02968849).[15,16]

One key hurdle for HIV-1 vaccine development is to elicit greater immune breadth to circulating strains of HIV-1.[9–13] To address the challenge of global HIV-1 diversity, we developed bioinformatically optimised bivalent global mosaic antigens that aim to expand immunological coverage of HIV-1 M group viruses.[17,18] To express mosaic Env and Gag-Pol immunogens, we used adenovirus serotype 26 (Ad26) vectors,[19] which differ substantially from Ad5 vectors in cellular receptor usage, tropism, innate inflammatory responses, adaptive immune phenotypes, and baseline neutralising antibody titres in human populations.[20] Phase 1 clinical trials with prototype Ad26 vectors expressing a single HIV-1 Env insert have shown induction of robust Env-specific immune responses in both peripheral blood and colorectal mucosa.[21–24]

Preclinical evaluations of HIV-1 vaccine candidates typically use simian immunodeficiency virus (SIV) or simian-human immunodeficiency virus (SHIV) challenge models in rhesus monkeys. Ad26 vectors expressing Env and Gag-Pol immunogens boosted with modified vaccinia Ankara (MVA) vectors expressing these immunogens showed partial protection against SIVmac251 and SHIV-SF162P3 challenges in rhesus monkeys.[25,26] Moreover, Ad26 vectors expressing these immunogens boosted with a purified SIV Env gp140 protein provided improved protection against heterologous SIVmac251 challenges.[27] These vaccines did not induce broad neutralising antibody responses, and correlates of protection were Env-specific binding and functional antiviral antibody responses, including antibody-dependent cellular phagocytosis (ADCP).[27,28]

A major limitation in the HIV-1 vaccine field to date has been the lack of direct comparability between preclinical studies and clinical trials, in terms of the vaccines, regimens, schedules, and assays used. We therefore aimed to evaluate the leading mosaic Ad26-based HIV-1 vaccine candidates in similarly designed preclinical and clinical studies to define the optimal HIV-1 vaccine regimen to advance into clinical efficacy trials.

Research in context

Evidence before this study

A safe and effective HIV-1 vaccine will most likely be required for a durable end to the HIV-1 pandemic. No licensed HIV-1 vaccine exists, and only four HIV-1 vaccine concepts have been evaluated for clinical efficacy to date. We developed a candidate HIV-1 vaccine consisting of priming with adenovirus serotype 26 (Ad26) vectors expressing bioinformatically optimised mosaic HIV-1 envelope (Env)/Gag/Pol immunogens and boosting with Ad26 vectors and adjuvanted Env gp140 protein. We evaluated this vaccine and others in parallel preclinical studies and phase 1/2a clinical studies. We searched PubMed throughout the study for published HIV-1 vaccine studies and ClinicalTrials.gov for ongoing HIV-1 vaccine clinical trials, and we found no evidence of previous testing of this vaccine candidate.

Added value of this study

All vaccines that were tested in this study showed favourable safety and tolerability profiles in humans. The mosaic Ad26/Ad26 plus gp140 HIV-1 vaccine induced robust humoral and cellular immune responses in both humans and rhesus monkeys. Immune responses in humans and rhesus monkeys were similar in magnitude, durability, and phenotype. This vaccine provided 67% protection against acquisition of six intrarectal simian-human immunodeficiency virus (SHIV)-SF162P3 challenges in rhesus monkeys.

Implications of all the available evidence

The mosaic Ad26/Ad26 plus gp140 HIV-1 vaccine met pre-established safety and immunogenicity criteria to advance into a phase 2b clinical efficacy study in sub-Saharan Africa, which is now underway (NCT03060629).

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Methods

APPROACH clinical study

Study design and participants

We did a multicentre, randomised, double-blind, placebo-controlled, phase 1/2a trial investigating the safety, tolerability, and immunogenicity of various vaccine regimens. These vaccine regimens contained Ad26.Mos.HIV (Ad26.Mos1.Env, Ad26.Mos1.Gag-Pol, and Ad26.Mos2.Gag-Pol), MVA-mosaic (MVA.Mos1 and MVA.Mos2), with or without gp140 protein (aluminium phosphate adjuvanted clade C gp140 Env protein). We recruited participants from 12 clinical sites in the USA, Rwanda, Uganda, South Africa, and Thailand. We included healthy, HIV-1-uninfected participants who were considered at low risk for HIV-1 infection and between the age of 18 years and 50 years. The protocol provides the full list of the inclusion and exclusion criteria. We obtained approval from the respective Institutional Review Boards at each clinical site and written informed consent from eligible participants.

Randomisation and masking

Following a 4-week screening period, we randomly assigned eligible participants to one of eight study groups: Ad26/Ad26 plus high-dose gp140, Ad26/Ad26 plus low-dose gp140, Ad26/Ad26, Ad26/MVA plus high-dose gp140, Ad26/MVA plus low-dose gp140, Ad26/MVA, Ad26/high-dose gp140, and placebo. Randomisation was stratified by region (ie, USA, Africa, and Asia) and was done by a computer-generated system (IWRS) and balanced by using randomly permuted blocks of size 8. We masked participants and investigators to treatment allocation throughout the study. Vaccines were provided in identical syringes, masked with blinding tape. The sponsor and the statistician were unmasked at the time of the primary analysis, which was done when all participants completed the week 28 visit or discontinued earlier.

Procedures

We primed participants at weeks 0 and 12 with Ad26.Mos.HIV (5×10^{10} viral particles per 0·5 mL) and gave them boosters at weeks 24 and 48 with one of the following combinations: Ad26.Mos.HIV with or without high-dose gp140 protein (250 μg) or low-dose gp140 protein (50 μg), MVA-mosaic (10^8 plaque-forming units per 0·5 mL) with or without high-dose or low-dose gp140 protein, or high-dose gp140 protein alone. Those in the control group received 0·9% saline at weeks 0, 12, 24, and 48. All study interventions were administered intramuscularly.

We followed up participants for up to 96 weeks during the clinic visits; follow-up is ongoing for most participants and we present results up to week 52. Blood samples for serum creatinine, aspartate transaminase, and alanine transaminase, haematology, and urinalysis were collected at several timepoints throughout the study. Baseline troponin was assessed at screening, and electrocardiograph (ECG) recorded both at screening and before the first boost vaccination at week 24.

Outcomes

Primary endpoints were safety and tolerability of the vaccine regimens and Env-specific binding antibody responses in each experimental group. The predefined timepoint for the primary analysis of safety and immunogenicity endpoints was week 28 (4 weeks after the third vaccination). Safety and immunogenicity were also assessed at week 52. Secondary endpoints were antibody effector function and cellular immune responses.

Local and systemic reactogenicity safety data were collected for 8 days after each vaccination. Unsolicited adverse events were analysed 28 days after vaccination. Data on serious adverse events and incident HIV-1 infections were collected during the entire study period (96 weeks).

Statistical analysis

The statistical analysis of safety data followed the intention-to-treat principle, including all participants who were randomly assigned to an intervention and received at least one vaccine dose or placebo. For each vaccine regimen, the number and proportion of participants with adverse events, serious adverse events, and laboratory abnormalities were tabulated by dose and over the entire regimen.

Immunogenicity data were analysed using the per-protocol immunogenicity population, comprising participants who received the first three vaccinations according to the protocol-specified vaccination schedule (plus or minus 2 weeks), and not diagnosed with HIV-1 infection before the primary endpoint at week 28. Immunogenicity data were analysed descriptively through tabulations of geometric mean with corresponding two-sided 95% CIs, or medians, but no formal statistical comparisons were made. Response rates and CIs for immunoassays were calculated as the number and proportion of participants meeting the predefined definition of response (ie, the cellular immune response). CIs were not adjusted for multiplicity.

This study is registered with ClinicalTrials.gov, number NCT02315703.

Rhesus monkey challenge study (NHP 13-19)

Study design and procedures

We immunised 72 Indian-origin rhesus monkeys (*Macaca mulatta*) using a similar study design as the APPROACH clinical study. We primed rhesus monkeys with Ad26.HIV.Mos (5×10^{10} viral particles per 0·5 mL) at weeks 0 and 12, and gave them boosters with the following regimens at weeks 24 and 52: Ad26.Mos.HIV with or without gp140 protein (250 μg), MVA-mosaic (10^8 plaque-forming units per 0·5 mL) with or without gp140 protein, or gp140 protein alone. Rhesus monkeys in the control group received 0·9% saline at

weeks 0, 12, 24, and 48. All study interventions were administered intramuscularly.

This study design allowed an evaluation of the immunogenicity and protective efficacy of these vaccine regimens in rhesus monkeys. All rhesus monkeys received intrarectal challenges once per week for 6 weeks with 500 50% tissue culture infectious dose ($TCID_{50}$) of the heterologous virus SHIV-SF162P3 starting at week 76 (ie, 6 months after completion of vaccination). Viral loads were evaluated weekly following challenge by a qualified viral load assay. Ad26 vectors and gp140 protein were produced at Janssen, the MVA vectors were produced at Walter Reed Army Institute of Research, and the SHIV challenge stock was produced in rhesus monkey peripheral blood mononuclear cells at Beth Israel Deaconess Medical Center. We obtained approval from the Institutional Animal Care and Use Committee.

Statistical analysis

Time-to-infection was analysed with Cox proportional hazard regression for discrete times, and final infection status was assessed by two-sided Fisher's exact test. Multiple comparison adjustments were done for the vaccine groups with a five-times Bonferroni adjustment.

To assess immune correlates of protection, assays were selected stepwise from a predefined set by cumulative logistic regression on time-to-infection using groups Ad26/Ad26, Ad26/gp140, and Ad26/Ad26 plus gp140 (appendix p 13). The prediction model was more powerful without the groups boosted with MVA, possibly related to the differential immune profiles of MVA vectors; therefore, these MVA boosted groups were excluded. Selection continued until no assay significantly improved the model, defined as $p<0·05$. For individual assays, Spearman correlations were calculated with time-to-infection (appendix p 13).

Role of the funding source

One of the study funders, Janssen, participated in data collection, data analysis, data interpretation, and writing of the report. DHB, FLT, FW, MGP, and HS had full access to all the data in the study. The decision to submit for publication was joint among all coauthors.

Results

Between Feb 24, 2015, and Oct 16, 2015, we randomly assigned 393 participants to receive at least one dose of study vaccine (figure 1A). At week 52, 39 (10%) of 393 participants prematurely discontinued from the study. Overall, 150 (38%) of 393 participants were from the USA, 129 (33%) from east Africa, 56 (14%) from South Africa, and 58 (15%) from Thailand. Of all participants, 212 (54%) were men and 219 (56%) were black or African American, 104 (26%) were white, 64 (16%) were Asian, and six (2%) were listed as other. Median age was 29 years (range 18–50) and median body-mass index was 24·8 kg/m² (15·6–51·8). No substantial demographic imbalances were seen between treatment groups (appendix p 14), and no difference in demographics was seen between participants receiving vaccines versus those receiving placebo.

Detailed evaluation of the safety and tolerability profile of these vaccine regimens is presented in the appendix (pp 15–20, 23–25). During the 8-day post-vaccination period, the most commonly reported solicited local adverse event was mild-to-moderate pain at the injection site (varying from 69% to 88% between the different active groups, after any dose, compared with 49% in the placebo group; appendix p 23). This adverse event generally decreased with subsequent vaccinations. Mild-to-moderate headache (46–65%), fatigue (44–70%) and myalgia (32–49%) were the most commonly reported solicited systemic adverse events (appendix p 24). Most adverse events reported during the 28-day reporting period after each vaccination were mild or moderate in severity. Five (1%) of 393 participants reported at least one grade 3 adverse event considered related to the vaccines: abdominal pain and diarrhoea (in the same participant), increased aspartate aminotransferase, postural dizziness, back pain, and malaise (appendix p 18). Overall, there was no remarkable difference in safety, tolerability, or reactogenicity between the seven different groups receiving vaccination. Additionally, one participant presented at the emergency department 12 h after receiving the first vaccination, describing signs and symptoms of an allergic reaction. Evaluation by the physician did not reveal signs indicative of an allergic reaction. The participant was discharged after receiving diphenhydramine, but without corticosteroids. This patient stated the episode resolved after 1 day. It was later discovered that the participant had a history of illicit drug use and bipolar disorder with hallucinations. This adverse event was considered as a severe allergic reaction possibly related to the vaccine (appendix p 18). Further vaccinations were discontinued for this participant. During the reporting period following each vaccination dose, no grade 4 adverse events or deaths were reported. Three incidental HIV infections occurred at a single site in South Africa (appendix p 7). Five more participants included in the week-52 analysis discontinued the study vaccination because of adverse events; one was considered related to the study vaccination (grade 1 urticaria), and four as unrelated (grade 1 chronic kidney disease, grade 3 lumbar vertebral fracture, grade 3 intraductal proliferative breast lesion, and grade 1 right bundle branch block). No safety concerns were identified following patient examination.

See Online for appendix

Overall, no substantial differences in safety or tolerability of any of the seven active vaccine groups were observed, considering solicited and unsolicited adverse events, grade 3 or 4 adverse events, serious adverse events, and adverse events leading to discontinuation.

All vaccine regimens were highly immunogenic. Binding antibody responses to autologous Env clade C gp140 were

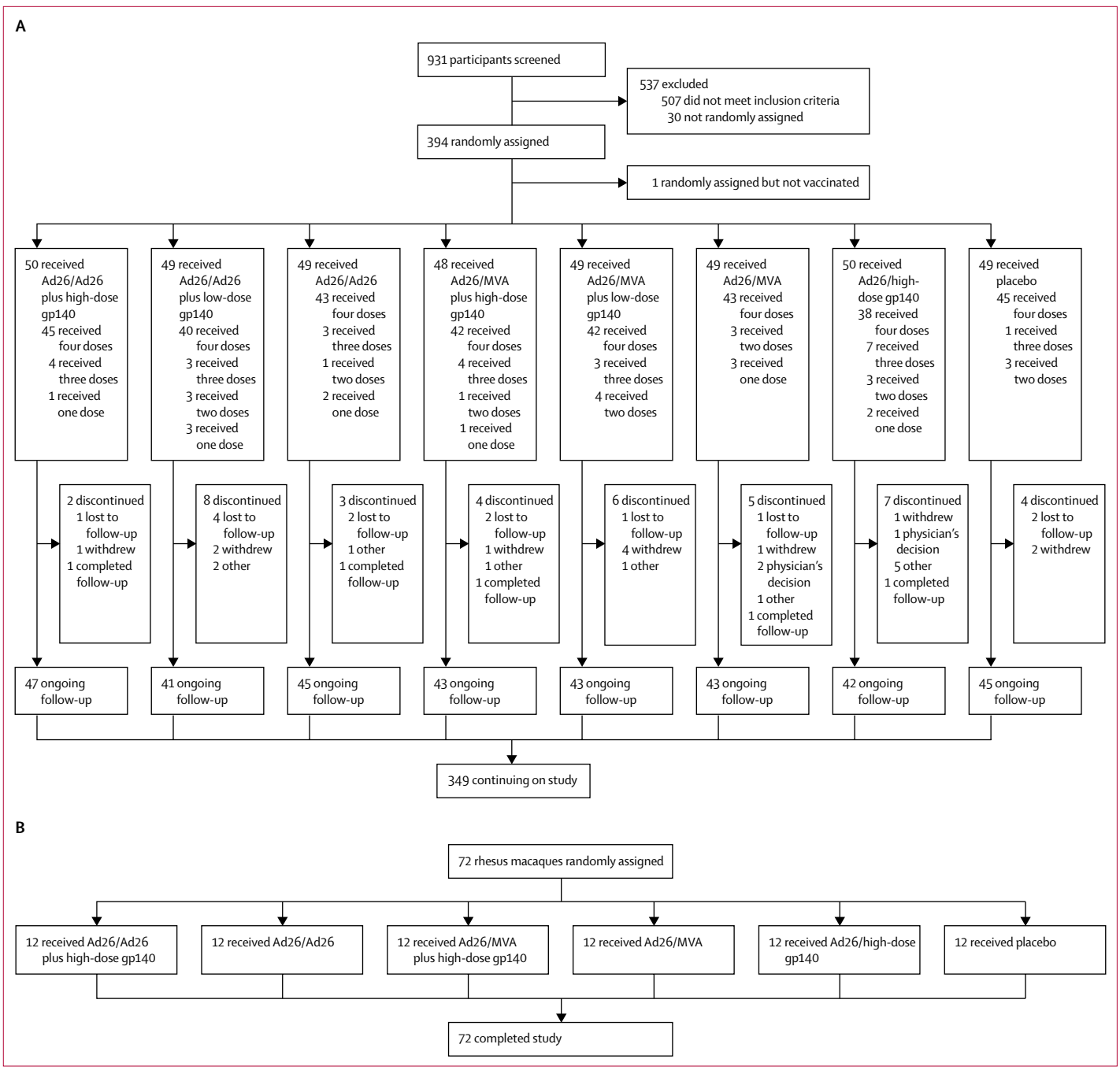


Figure 1: **Trial profile**
(A) APPROACH clinical study. (B) Rhesus monkey challenge study (NHP 13-19). Ad26=adenovirus serotype 26. MVA=modified vaccinia Ankara.

detected in all vaccinees evaluated following the second immunisation at week 12 (100%, 95% CI 93–100). Responses were differentially boosted after the third vaccination at week 24 and fourth vaccination at week 48. After the week 24 vaccination, most groups maintained 100% antibody response (figure 2A). ELISA titres were higher in the groups that received the gp140 protein boost than in those that did not and were dependent on the gp140 dose. Inclusion of either Ad26 or MVA vector in the boost also increased responses. Total IgG responses to cross-clade founder Envs, to Envs isolated from chronically infected individuals, and to consensus Envs were similar to the autologous responses, demonstrating binding to multiple global Envs (appendix pp 26–28). 33 (80%) of 41 participants in the group that received Ad26/Ad26 plus high-dose gp140

also showed responses to gp70-V1V2 from Env 1086C (appendix pp 27, 28). IgG subclasses were primarily IgG1 and IgG3, with minimal to no induction of IgG2 and IgG4 (appendix pp 27–29).

Antibody functionality was evaluated by ADCP assays (figure 2B) and correlated with the binding antibody responses (appendix p 30). ADCP responses were strongest in the vaccinees who received the protein boost, and the magnitude of responses increased with protein dose and presence of vector. In the Ad26/Ad26 plus high-dose gp140 boost group, 34 (72%) of 47 participants exhibited ADCP responses at week 28, and 36 (80%) of 45 participants did so at week 52. Serum neutralising activity was only detected against easy-to-neutralise tier-1 HIV-1 variants (appendix pp 21, 31).

High frequencies of cellular immune responses were detected by interferon-γ enzyme-linked immunospot (ELISPOT) assays against Env potential T-cell epitope $(PTE)_g$ peptide pools (figure 2C) and against vaccine-matched peptide pools[29] (appendix pp 32–34). ELISPOT responses to Env peptide pools increased in groups that received the protein boost. In the Ad26/Ad26 plus high-dose gp140 group, 36 (77%) of 47 participants exhibited ELISPOT responses at week 26, and 34 (83%) of 41 participants did so at week 50. ELISPOT responses were also detected against Gag and Pol peptide pools (appendix pp 32–34). Intracellular cytokine staining for interferon γ or interleukin 2 showed that CD4 and CD8 T-cell responses were both generated; CD4 T-cells were directed primarily to Env whereas CD8 T-cells were directed primarily to Pol and to a lesser extent to Gag and Env (appendix pp 35–40).

The breadth of T-cell responses was assessed in 20 participants from the Ad26/Ad26 plus high-dose gp140 group (n=10) and Ad26/MVA plus high-dose gp140 group (n=10) after the first boost by ELISPOT assays using PTE_g and vaccine-matched subpools consisting of ten peptides. A median of nine subpools (range 6–28) were recognised in the Ad26/Ad26 plus high-dose gp140 group and ten subpools (range 1–17) in the Ad26/MVA plus high-dose gp140 group, reflecting a conservative estimate of T-cell breadth induced by the vaccines (figure 2D).

Most individuals in sub-Saharan Africa had low-to-moderate titres of baseline Ad26-specific neutralising antibodies (appendix p 41), consistent with previous epidemiological surveys.[23] These titres were substantially lower than Ad5-specific neutralising antibodies titres in these populations.[30] No associations were observed between baseline Ad26-specific neutralising antibodies before immunisation and ELISA or ELISPOT responses following immunisation, showing that these vector-specific antibody titres did not interfere with the vaccine immune responses (appendix p 42). The appendix (pp 43–45) shows the variability in immune response to vaccine stratified by sex, age, or region.

We randomly assigned 72 rhesus monkeys to receive one of five different vaccine regimens or placebo (12 rhesus monkeys per group; figure 1B). Binding antibody responses against clade C Env were detected in all vaccinated monkeys by ELISA (figure 3A), and regimens that included the protein boost exhibited substantially higher titres. Antibody titres declined from peak (at week 54) to the day of challenge (at week 76; appendix p 46). Functional ADCP responses were detected after the heterologous boost immunisations (figure 3B) and correlated with binding antibody titres (appendix p 30). Serum neutralisation of tier-1A viruses was observed at the peak of the immune response, while serum neutralisation titres for tier-1B viruses were low (appendix pp 47, 48), and no neutralisation of primary isolate-like tier-2 viruses was observed.

Cellular immune responses against HIV-1 Env, Gag, and Pol were detected by interferon-γ ELISPOT assays using both PTE_g peptide pools (figure 3C; appendix p 49) and vaccine-matched peptide pools (appendix pp 50, 51). Regimens that included the MVA boost immunisations showed the highest mean ELISPOT responses. These trends were confirmed by frequencies of interferon-γ or interleukin-2 producing CD4 and CD8 T-cells enumerated by flow cytometry (appendix pp 52, 53).

All animals were then challenged six times by the intrarectal route with the heterologous, tier-2 neutralisation-resistant virus SHIV-SF162P3. Rhesus monkeys in the placebo group were infected after a median of one challenge (range 1–5). The various vaccine regimens showed different degrees of protective efficacy, defined as reduced per exposure acquisition risk and reduced numbers of infected monkeys after the full series of challenges as compared with the control group. The Ad26/Ad26 plus gp140 regimen provided the greatest protection compared with the other vaccine regimens, with eight (67%) of 12 rhesus monkeys uninfected after the challenge series (figure 4A). This effect corresponded to a 94% reduction in exposure acquisition risk (log-rank test $p=0\cdot001$) and 67% complete protection (two-sided Fishers' exact test $p=0\cdot007$). The other regimens showed lower point estimates of protection in this model.

On the basis of previously reported potential correlates of protection, we selected 16 humoral and cellular immunological assays to generate an immune readout-based prediction model (appendix p 13). The model of immune correlates that best predicted time-to-infection included clade C ELISA and PTE_g Env ELISPOT responses at week 28 (model fit of both assays $p=0\cdot001$; figure 4B; appendix p 13), and the linear predictor defined with these two assays strongly correlated with observed data (Spearman correlation $\rho=0\cdot55$). None of the other immunological measures significantly improved the predictive accuracy of this model once these two readouts were included.

Given the parallel design of the studies in humans and rhesus monkeys, and the comparable performance of the clade C ELISA (appendix p 54) and Env PTE_g ELISPOT assays, we did a post-hoc comparison of the vaccine-elicited immune responses in rhesus monkeys and

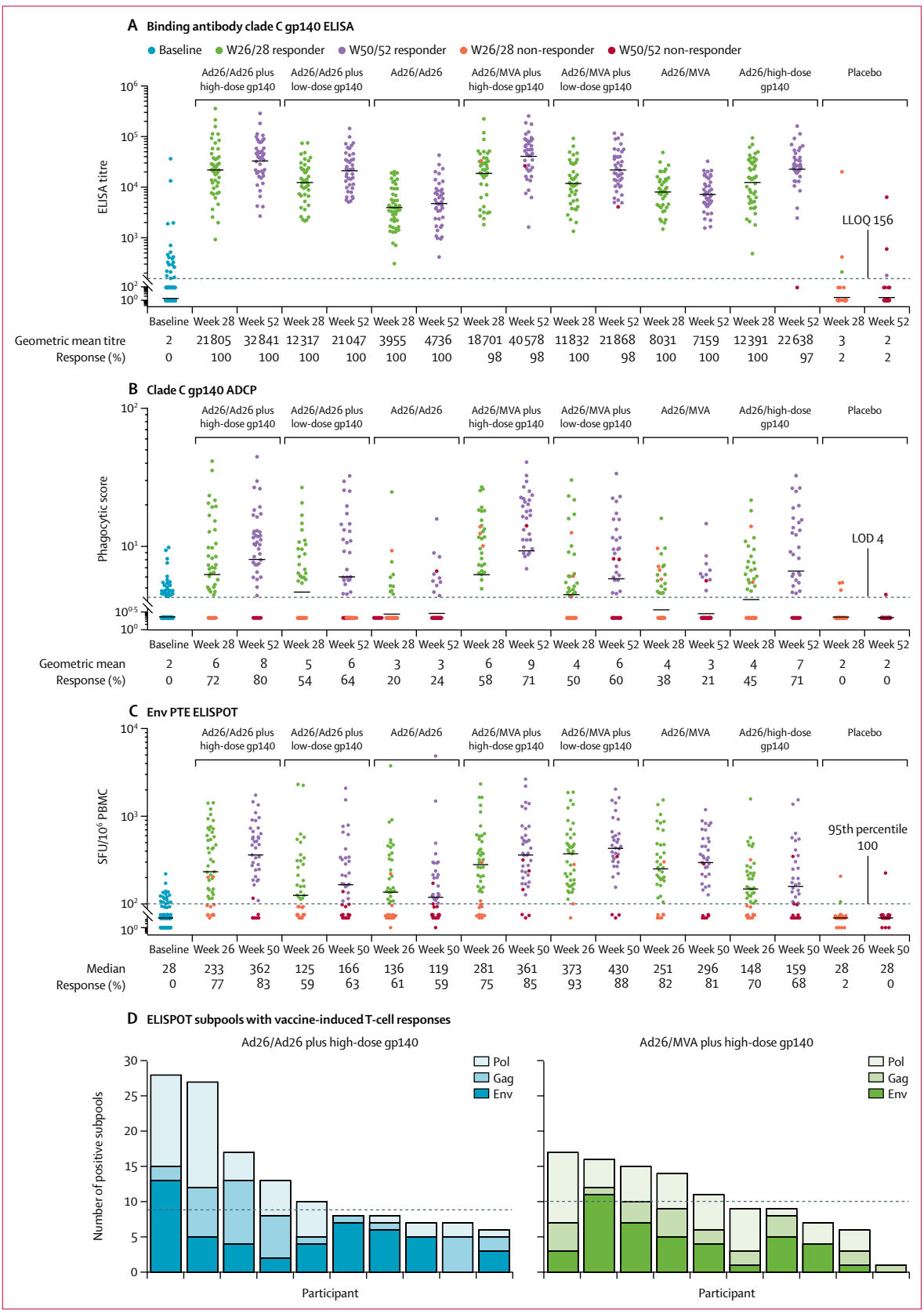


Figure 2: **Immune response to vaccination regimens in humans**

Responder rates are shown for each vaccine group at baseline, after the third vaccination at weeks 26 or 28, and fourth vaccination at weeks 50 or 52. Vaccine response was defined as value more than threshold (if baseline is <threshold or is missing); otherwise, it was defined as value with a three-time increase from baseline (if baseline is ≥threshold). (A) The dotted line is the LLOQ threshold. (B) The dotted line is the LOD threshold. (C) The dotted line is the 95th percentile of the overall baseline values. (D) Number of ELISPOT subpools with vaccine-induced T-cell responses for a subset of participants in Ad26/Ad26 plus high-dose gp140 and Ad26/MVA plus high-dose gp140 vaccine groups. The dotted line is the median number of subpools recognised. W26/28=weeks 26 or 28. W50/52=weeks 50 or 52. Ad26=adenovirus serotype 26. MVA=modified vaccinia Ankara. LLOQ=lower limit of quantification. ADCP=antibody-dependent cellular phagocytosis. LOD=limit of detection. ELISPOT=enzyme-linked immunospot. Env=envelope. PTE=potential T-cell epitope. PBMC=peripheral blood mononuclear cells. SFU=spot forming units.

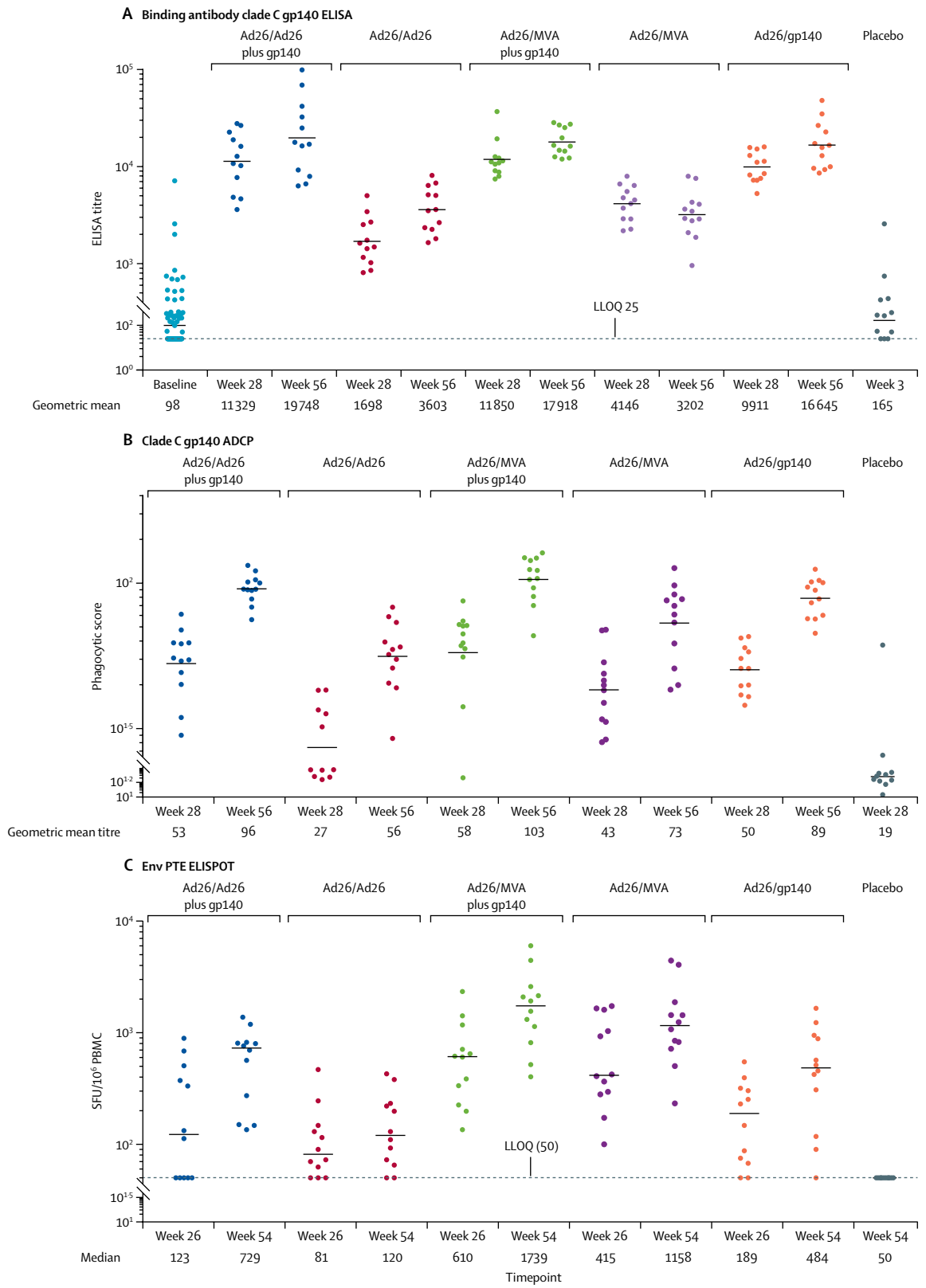
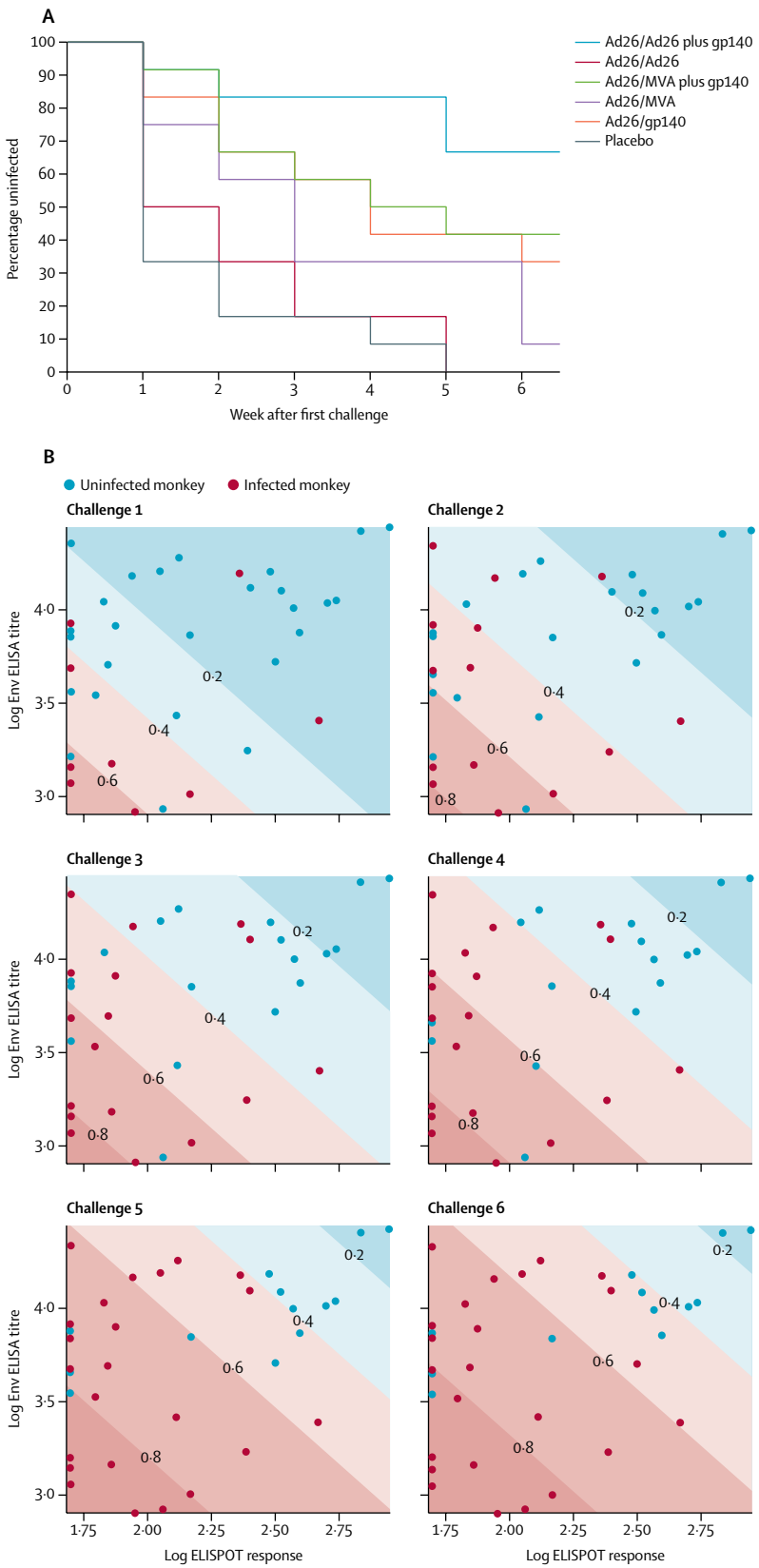


Figure 3: **Immune response to vaccination regimens in rhesus monkeys**
Responses are shown for each vaccine group at baseline, after the third vaccination at weeks 26 or 28, and fourth vaccination at weeks 54 or 56. Vaccine response was defined as value more than threshold (if baseline is <threshold or is missing); otherwise, it was defined as value with a three-time increase from baseline (if baseline is ≥threshold). The dotted lines are the LLOQ thresholds. Ad26=adenovirus serotype 26. MVA=modified vaccinia Ankara. LLOQ=lower limit of quantification. ADCP=antibody-dependent cellular phagocytosis. Env=envelope. PTE=potential T-cell epitope. ELISPOT=enzyme-linked immunospot. PBMC=peripheral blood mononuclear cells. SFU=spot forming units.



humans. The rhesus monkey and human ELISA data were compared for each immunisation regimen, and ranking between regimens were analogous between rhesus monkeys and humans (figure 5A). When antibody titres were compared longitudinally between species, similar kinetic profiles were observed with a slightly faster decrease of antibody titres in rhesus monkeys than in humans (figure 5B). A comparison of ELISPOT responses between rhesus monkeys and humans was less clear (figure 5C) and showed earlier induction of cellular immune responses in humans than in rhesus monkeys (figure 5D). Moreover, the ADCP (figures 2B, 3B) and intracellular cytokine staining data (appendix pp 35–40, 52, 53) suggested similarities in antibody and T-cell functionality for both species. These data suggest substantial comparability in the magnitude, kinetics, durability, and phenotypes of immune responses induced by these vaccines in humans and rhesus monkeys.

To support the initiation of a phase 2b efficacy study (NCT03060629), go or no-go criteria based on the immunological correlates of protection in rhesus monkeys were established in advance (appendix p 22). These criteria were set on the basis of frequency and magnitude of the cellular and humoral immune responses associated with protection in rhesus monkeys. Both the Ad26/Ad26 plus high-dose gp140 and Ad26/MVA plus high-dose gp140 groups achieved these criteria in humans, and the remaining regimens were down-selected. In particular, the regimens that included low dose or no gp140 protein did not generate sufficient ADCP responses to meet these criteria. A comparison of Ad26/Ad26 plus high-dose gp140 and Ad26/MVA plus high-dose gp140 across humoral and cellular immune responses showed no superiority for either regimen. The higher point estimate of protective efficacy in rhesus monkeys as well as regimen simplicity and manufacturability favoured selection of the Ad26/Ad26 plus high-dose gp140 regimen.

Discussion

We showed that mosaic Ad26-based HIV-1 vaccine regimens were well tolerated and induced robust humoral and cellular immune responses in healthy individuals in east Africa, South Africa, Thailand, and the USA. All vaccine regimens tested were safe and generally well tolerated. There were no remarkable differences between the different active groups in terms of solicited or

Figure 4: **Protection and correlates in rhesus monkeys**
(A) Kaplan-Meier plot of the protection of each vaccine regimen in rhesus monkeys, assessed 1 week after each challenge. No animals were censored. (B) Humoral and cellular immune response measured by clade C ELISA at week 28 and PTE_9 Env ELISPOT at week 26, and the infection status the week following each of six challenges (at weeks 77–84) of rhesus monkeys from the following groups: Ad26/Ad26, Ad26/gp140, Ad26/Ad26 plus gp140. The diagonal lines display model-derived probabilities of infection, modelled on ELISA and ELISPOT responses. Ad26=adenovirus serotype 26. MVA=modified vaccinia Ankara. Env=envelope.

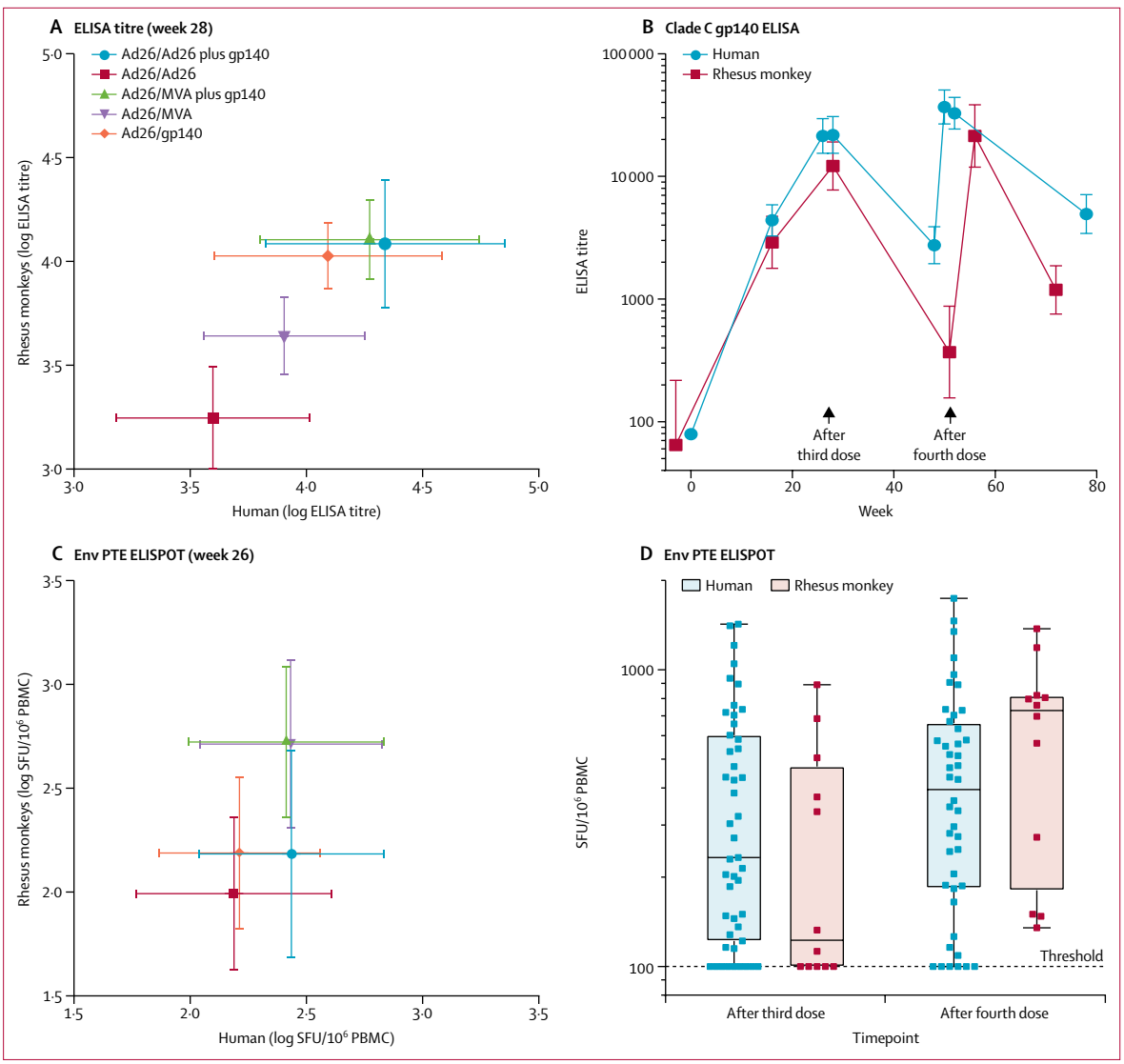


Figure 5: **Data comparison of humans and rhesus monkeys**

Data are geometric mean titres or SFU per million PBMC. Panels (B) and (D) compare the Ad26/Ad26 plus high-dose gp140 regimen. Error bars are SDs in panels (A), (C), and (D), and 95% CIs in panel (B). Comparisons of the magnitude of immunological responses between rhesus monkey and human studies are shown. Rhesus monkey ELISA data in (A) and (B) have been transformed to human ELISA units. Ad26=adenovirus serotype 26. MVA=modified vaccinia Ankara. PBMC=peripheral blood mononuclear cells. SFU=spot forming units. Env=envelope. PTE=potential T-cell epitope. ELISPOT=enzyme-linked immunospot.

unsolicited adverse events, including grade 3 or 4 adverse events, serious adverse events, adverse events leading to discontinuation, or laboratory-related or ECG-related adverse events. These vaccine regimens also elicited largely comparable immune responses in rhesus monkeys and provided substantial protection against repetitive, heterologous, intrarectal SHIV-SF162P3 challenges. In both humans and rhesus monkeys, the optimal vaccine regimen included priming with Ad26 vectors expressing mosaic HIV-1 Env and Gag-Pol immunogens and boosting with the combination of Ad26 vectors and high-dose Env gp140 protein. The immunogenicity data met pre-established criteria to initiate a phase 2b efficacy trial, called Imbokodo (HPX2008/HVTN 705; NCT03060629), which will evaluate the protective efficacy of this vaccine concept against acquisition of HIV-1 infection in 2600 young women in southern Africa.

Previous HIV-1 vaccine candidates have typically been limited to specific regions of the world.[11,13,14] Optimised mosaic antigens[17,18] offer the theoretical possibility of developing a global HIV-1 vaccine. Cellular immune breadth induced by these mosaic Ad26-based vaccine candidates (median of nine to ten epitopes) was substantially greater than that reported previously for other Ad5-based and Ad26-based vaccines expressing natural sequence antigens (median of one epitope; appendix p 55).[31,21] Therefore, responses to the mosaic vaccine might have enhanced potential to recognise

circulating virus strains.[32,33] Although the mosaic antigens were initially designed to improve T-cell breadth, these immunogens also elicited cross-clade binding antibodies to multiple HIV-1 Env antigens.

In rhesus monkeys, the statistical correlates of protection included antibodies against clade C Env as measured by ELISA and T-cell responses as measured by Env PTE_g ELISPOT assays. These two parameters were combined into a linear predictor that correlated with protective efficacy in this animal model and formed the basis of the criteria to advance the mosaic Ad26/Ad26 plus high-dose gp140 HIV-1 vaccine candidate into a clinical efficacy trial. We speculate that these immunological parameters might be surrogate markers for actual protective immune responses, which probably involve functional antibody responses.[26,27] The role of virus-specific T-cell responses in protecting against acquisition of infection remains to be determined.

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The principal limitation of this study is that the relevance of vaccine protection in rhesus monkeys to clinical efficacy in humans remains unclear. As such, the preclinical challenge models might need to be refined when clinical efficacy data become available. Another limitation is a lack of knowledge of a true mechanistic correlate of protection against HIV-1 in humans. The statistical correlates identified in this study have practical use, but further investigation is required to define the actual mechanisms of protection.

In conclusion, we demonstrated that the mosaic Ad26/Ad26 plus high-dose gp140 HIV-1 vaccine was comparably and robustly immunogenic in humans and rhesus monkeys, and it showed substantial protective efficacy in rhesus monkeys. A phase 2b clinical efficacy trial has been initiated in southern Africa to determine whether this vaccine concept will prevent HIV-1 infection in humans.

Contributors

DHB, FLT, FW, MGP, and HS led the study and did the primary manuscript writing. DHB, FW, DJS, GA, MLR, NLM, LP, JPN, ENB, AC, DJ, KES, WL, KF-dB, DvM, JV, RZ, JT, JH, ZE, MGP, and HS did the preclinical study design, study execution, data analysis, and manuscript editing. DHB, FLT, DJS, GA, MLR, NLM, KES, BK, GDT, DCM, GG, NF, MJM, LB, JJ, JH, ES, EK, HK, JM, NG, KM, KC, FP, EL, FL, SN, PP, SB, TC, RF, GL, CB, KC, RR, JS, LS, MW, DvM, RZ, LL, SN, JT, JH, ZE, MGP, and HS did the clinical study design, study execution, and data analysis, and manuscript editing. All coauthors provided a full review of the Article, are fully responsible for all content and editorial decisions, were involved in all stages of manuscript development, and have approved the final version.

Declaration of interests

DHB has received grant funding from the National Institutes of Health, the Bill & Melinda Gates Foundation, and Janssen Vaccines & Prevention BV. DHB is a coinventor on HIV-1 vaccine antigen patents that have been licensed to Janssen Vaccines & Prevention BV. FLT, FW, DJS, CB, KC, RR, JS, LS, MW, KF-dB, DvM, JV, RZ, LL, SN, JT, JH, ZE, MGP, and HS are employees of Janssen, pharmaceutical companies of Johnson & Johnson. LL is a consultant to Janssen, pharmaceutical companies of Johnson & Johnson. All other authors declare no competing interests.

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